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Effects of litter mixing on litter decomposition and soil properties along simulated invasion gradients of non-native trees

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Running title: Litter mixing effects along invasion gradients

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Abstract

Aims: Plant invasions generally lead to mixtures between native and non-native litter. We assess the interactions between leaf litters from two invasive non-native trees (*Robinia pseudoacacia* or *Ailanthus altissima*) and the native *Populus alba* on litter decomposition, nutrient release and soil properties along two gradients of invasion. **Methods:** Microcosms with field-collected soil covered by varying proportions of native and non-native litter simulated the two invasion gradients. We assessed the proportion of remaining litter mass and nutrient mass (N, P, C), and soil C, N-NO₃⁻, total N, and pH, five times throughout a period of 11 months. Observed values were compared to the expected values on the assumption of no interactions. **Results:** Litter mass and C mass decayed slower in *Robinia* and faster in *Ailanthus* than in *Populus*. The three species immobilized N and P. Soil properties did not differ across pure litters. Both litter mixture gradients showed additive or antagonistic interactions on litter decomposition, whereas N and P mass were equal or higher than expected. The proportion of non-native litter in the mixture had non-linear effects on most variables, suggesting that the impact of these non-native trees on litter decay levels off or even declines as they become more abundant. **Conclusion:** The impacts of *Ailanthus* and *Robinia* litter on soil processes should not be derived from single species experiments, both due to non-additive effects and to non-linear responses to litter abundance.

Introduction

Litter decomposition is a key process for nutrient cycling in forest ecosystems, and thus for primary production (Berg and McClaugherty 2014; Hickman et al. 2013; Vogt 1991). The litter decomposition process is controlled by climatic and edaphic factors, by the nature of the soil

community, and by the quality of the litter (Aerts 1997; Berg and McClaugherty 2014; Gallardo and Merino 1993; Jo et al. 2016). Thus, invasion of ecosystems by non-native plants with litter properties different from those prevailing in the native community may alter the nutrient cycle and soil properties (Castro-Díez et al. 2014a; Ehrenfeld and Scott 2001; Incerti et al. 2018; Kourtev et al. 2002). Impacts may be dramatic when the invaders represent novel functional key traits and/or when they are very abundant (Chapin et al. 1996; D'Antonio and Corbin 2003; Vitousek and Walker 1989).

One of the most widely described impacts of invasive plants is that of the litter on soil properties and processes (Castro-Díez et al. 2014a; Ehrenfeld and Scott 2001; Hulme et al. 2013; Liao et al. 2008; Pyšek et al. 2012). Overall, invasive plants tend to be more productive than natives (Pyšek and Richardson 2007; van Kleunen et al. 2010) and to produce litters with properties associated with a fast decomposition (e.g. high nutrient content, high specific leaf area (SLA), low lignin and phenolic content) (Castro-Díez et al. 2014a; Ehrenfeld 2003; Pyšek et al. 2012). However, exceptions to this trend can be often found (Godoy et al. 2010; Marchante et al. 2019; McArthur et al. 1994; Nilsson et al. 1999), and recent studies suggest that this general trend may be the result of a biased selection of the non-native species with the largest impacts on ecosystem processes (Incerti et al. 2018; Jo et al. 2016).

The pattern of non-native tree invasion over space and time generally leads to litter mixtures between native and non-native species (Chen et al. 2013; Gartner and Cardon 2004; Hickman et al. 2013). Yet, most studies aiming to assess the impact of this litter on soil processes rely on comparisons between situations with pure native litter and pure non-native litter (Castro-Díez et al. 2009; Godoy et al. 2010; Incerti et al. 2018). Thus, these studies implicitly assume that native and non-native litters do not interact with each other, so that the dynamics of the litter mixtures might be derived from the dynamics of isolated litters knowing their proportions in the mixture. This situation is referred to as “additive effects” between litters, i.e. the whole is the sum of the components. Yet, the growing number of studies addressing the dynamics of litter mixtures highlights that non-additive effects are more frequent than additive effects and that positive effects or synergies (i.e. faster decomposition –or lower litter mass remaining– than predicted from component species decaying alone) are more frequent than negative or antagonistic effects (Gartner and Cardon 2004; Hattenschwiler et al. 2005; Lecerf et al. 2011). Mechanisms behind interactions between litters in mixtures include the transfer of nutrients or other compounds across litters, increased habitat complexity in litter mixtures, enhancing consumer diversity (Chen et al. 2013; Gartner and Cardon 2004; Lecerf et al. 2011; Zhang et al. 2014), or feedbacks between edaphic organisms and the environment (Elgersma and Ehrenfeld 2011).

Another widespread assumption is that invaders impacts are proportional to the invaders density, i.e. that the per-capita impact is constant (Parker et al. 1999). Yet, the few studies that specifically address the impact-density relation showed a variety of responses, including threshold effects (i.e. no impact below certain threshold density) (Elgersma and Ehrenfeld 2011; Maron and Marler 2008; Thiele et al. 2010). Given that most studies supporting impact assessment are focused on high-density invasions (Elgersma and Ehrenfeld 2011; Strayer et al. 2006; Yokomizo et al. 2009), the assumption of per-capita constant impact, if false, may involve high management costs (Yokomizo et al. 2009).

In spite of recent advances and reviews, the dynamics of litter mixtures have revealed to be complex and difficult to predict. For instance, non-additive effects of litter mixtures have been suggested to be greater when component litters differ greatly in functional traits (Wardle et al. 1997), but empirical evidences provide mixed support to this hypothesis (Chen et al. 2013; Hoorens et al. 2003; Lecerf et al. 2011; Quested et al. 2002). Length of the decay period may also affect the sign of the interactions (Chen et al. 2013; Gartner and Cardon 2004; Lecerf et al. 2011). Finally, the proportion of different litters in the mixture may also interfere with the strength of the interactive effect (Gartner and Cardon 2004; Scowcroft 1997; Zhang et al. 2014). In this line, similar proportions of litters with different traits in mixtures have been suggested to promote non-additive effects on litter decomposition (Chen et al. 2013). However, few studies evaluate the effects of mixtures with different proportions of litters (Gartner and Cardon 2004, but see Elgersma and Ehrenfeld 2011; Scowcroft 1997). Thus, there is an urgent need to fill these gaps with empirical knowledge to prevent managers to adopt scarcely tested assumption (as the constant per-capita impact) that may lead to implementing ineffective management actions (Sofaer et al. 2018; Yokomizo et al. 2009).

In Europe, including Spain, riparian forests, along with coastal ecosystems, are the most invaded natural terrestrial habitats (Chytrý et al. 2008; Vilà et al. 2001). Riparian forests are particularly prone to invasion because they match many of the conditions hypothesized to increase invasion success (Castro-Díez and Alonso 2017; Catford et al. 2011; Catford et al. 2009; Hood and Naiman 2000; Tabacchi et al. 2005; Tickner et al. 2001): 1) they receive intermittent pulses of resources thanks to periodic floods; 2) floods reduce the biotic resistance of the native community and create gaps that can be readily invaded by any pioneer species; 3) abiotic conditions are mild, thanks to the climatic buffering exerted by the tree canopy and the presence of a permanent water table; 4) rivers and floodplains are corridors for species propagules—either native or non-native—; 5) land-uses nearby rivers (agriculture, urbanization) increase propagule pressure of non-native plant species; 6) altered hydrologic regimes change the abiotic context where native species were selected and create opportunities for newly-arrived species.

In the inner Iberian Peninsula, riparian forests have been invaded by different non-native tree species (Cabra-Rivas and Castro-Díez 2016; Castro-Díez et al. 2009; Medina-Villar et al. 2015b). Two of the most common are *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. These species now coexist with dominant native trees, such as *Populus alba* L., *Populus nigra* L., *Fraxinus angustifolia* Vahl or *Ulmus minor* Mill., leading to litter mixtures between native and invasive trees.

In this study, we address the question of how the non-native litter of *A. altissima* and *R. pseudoacacia* alter nutrient cycling and soil properties. Previous field and lab experiments suggest that *A. altissima* litter decomposes faster than that of coexisting natives, while *R. pseudoacacia* litter does the opposite (Castro-Díez et al. 2009; Medina-Villar et al. 2015a). Under field conditions, *A. altissima* was found to decrease soil total N and *R. pseudoacacia* to increase soil mineral N (ammonium and nitrate) as compared to non-invaded forests (Medina-Villar et al. 2016). By contrast, a microcosm experiment revealed no difference in the effects of native and non-native litter on several soil properties (Castro-Díez et al. 2012). However, to our knowledge

there is no information on how the litter of these two species interferes with the decomposition of native litter, or on the relationship between the abundance and the impact of the invaders.

Specifically, we address the following questions. 1) How do litter decomposition dynamics differ between the two invasive trees and a coexisting native tree? 2) Are there non-additive positive or negative effects of the non-native litter on litter decomposition and soil properties? 3) How do these effects vary through incubation time? 4) How do these effects vary along a simulated gradient of invasion (increasing proportion of non-native litter)?

Materials and Methods

Study species

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) is native to South East Asia, while *Robinia pseudoacacia* L. (Fabaceae) is native to the Appalachian Mountains in Eastern North America. Both are fast-growing deciduous trees with compound leaves. They were introduced as ornamental plants in Europe, in the 18th and the 17th century, respectively (Sanz Elorza et al. 2004). Now they are considered as global invaders, being among the 100 worst invasive species in Europe (Basnou 2009; Basnou and Vilà 2009). In Spain these species are widespread in floodplains, borders of crops and roads, and (sub)urban areas (Sanz Elorza et al. 2004). Their fast growth, high seed production and profuse resprout ability, along with the N-fixing capacity of *R. pseudoacacia*, and the efficient dispersal of *A. altissima* seeds by wind and water, have been listed among the causes explaining their invasive success (Cabra-Rivas and Castro-Díez 2016; Cierjacks et al. 2013; Kowarik and Saumel 2007). The native tree *Populus alba* L. (Salicaceae) has been selected as control species due to its high dominance in the riparian forest of middle-low altitudes and basic soils of inner Spain (Lara et al. 1996). It is a deciduous, simple leaves tree, which produces large amounts of tiny, wind-dispersed seeds (Prada and Arizpe 2008). For simplicity, we name the species by the genus name from now on.

Leaf litter sampling

The leaf litter sampling was performed in the riparian forest of the mid-low Henares River (or its subsidies) along a stretch of 15 km, between the municipalities of Cabanillas del Campo (40°38'04"N, 3°14'06"W) and Los Santos de la Humosa (40°30'58" N, 3° 17' 06"W), both in Guadalajara province (central Spain). In this stretch, four locations were selected (Cabanillas del Campo, Alovera, Chiloeches and Los Santos de la Humosa) and litter from each species was collected in one (*Populus*), two (*Ailanthus*) or the four locations (*Robinia*). The altitude ranges from 590 to 660 m.a.s.l. The climate is Mediterranean continental, with cold winters and arid summers. The mean annual temperature is 13.5°C and the mean annual precipitation is 358 mm (weather station of Alcalá de Henares-El Encín, 600 m.a.s.l., period 1970–2009). Soils are Calcic Cambisols + Calcic Regosols or Calcic Fluvisol + Calcic Cambisol + Gleyo-calcic Fluvisols (FAO/IIASA/ISRIC/ISSCAS/JRC 2012).

Senescent leaves (those which detach with a gentle touch) were collected at the time of natural abscission (October-December 2015) from a minimum of 10 trees per species and location. Sampling trees were at least 15 m apart from each other to assure that they were different

genets. Once in the lab leaf litter was pooled by species, air-dried and stored in paper bags until being used. Given that leaflets and rachises of *Ailanthus* and *Robinia* tend to fall separately, we selected 10 full leaves (with rachis and all leaflets) to get the air-dried-weight proportion of rachis and lamina. This proportion was kept for subsequent chemical analysis and experimental setup.

From each pool of leaf litter, five pseudoreplicates of ca. 4 g of air-dried weight (keeping the rachis:lamina proportion in compound leaves) were selected; they were oven dried (60°C >48 h) to obtain the dry weight, and subsequently grounded to a particle size of 1 mm in a Culatti mill for chemical analyses. The water content of these samples was used to correct the air-dried weight of the leaf litter in the experimental setup (see below).

Soil sampling

In December 2015 we collected topsoil from 0 - 20 cm depth (after removing the litter layer) in the Henares River floodplain in the location of Los Santos de la Humosa, where vegetation was dominated by *Populus alba* and no non-native tree was present. Stones and large debris were removed. Soil was air-dried and passed through a 2 mm-sieve to remove large particles and homogenized. Five 350 g soil samples were separated, dried in the oven (60°C ≥ 72 h) and kept in the freezer at -20°C for subsequent chemical analyses.

Soil moisture calibration

Prior to the beginning of the experiment we conducted a soil moisture – soil weight calibration in order to monitor soil moisture during the experiment. Six previously weighed 500 cm³ (12 x 9 x 5 cm) polypropylene containers with five drainage holes in the bottom were filled with 250 g of air-dried experimental soil. Containers were then kept in larger aluminum trays with 3 cm of water for several days until soils were fully saturated. Then containers were left to freely drain until they stopped dripping from the bottom. At that point, containers were weighed, left to air dry in the lab, and re-weighed one to four times per day, until the soil started to show cracks and to lose its structure. Finally, containers were oven-dried at 60°C for ≥ 72 h and weighed. Soil moisture was calculated for every weighing time as the proportion of soil water (full container weight minus empty container weight minus dry soil weight) with respect to the water content at soil field capacity (Castro-Díez et al. 2012). The relation between container weight and soil moisture was used to calculate the amount of water required to keep experimental containers at 70-90% of soil field capacity during the experiment.

Experiment setup

The experiment was set up on 28th January 2016. We constructed microcosms by filling 162 polypropylene containers like those used for soil moisture calibration with 250 g of air-dried homogenized soil (ca. 3 cm of soil depth). The soil of each container was directly covered with ca. 3.5 g of previously-weighed air-dried leaf litter, corresponding to one of the following treatments: 100% *Populus*, 100% *Ailanthus*, 100% *Robinia*, 75:25 *Ailanthus:Populus*, 50:50 *Ailanthus: Populus*, 25:75 *Ailanthus: Populus*, 75:25 *Robinia:Populus*, 50:50 *Robinia: Populus*, and 25:75 *Robinia: Populus*. In this way, we simulated two invasion gradients of the riparian native forest (dominated by *Populus*) by each invasive tree. The quantity of leaf litter used was

equivalent to 324 g m^{-2} , which falls within the range of annual leaf litter production reported in the literature for these species in monospecific stands: 305 g m^{-2} for *Ailanthus* (González-Muñoz et al. 2013), 310 g m^{-2} for *Robinia* (Tateno et al. 2007) and 412 g m^{-2} for *Populus* (Medina-Villar et al. 2015b). We did not use litter bags, as usually done in this type of experiments, to maximize the contact between leaf litter and soil, as occurs in the field. However, this approach has the disadvantage of an increasing difficulty to separate the remaining litter from the soil – and thus an increasing variability – as decomposition proceeds. To compensate for this drawback, we doubled the number of replicates in the last harvest, as explained below.

Eighteen containers (replicates) per treatment were prepared. Groups of four or six replicates were randomly placed in each of four drainage aluminum trays with ca. 2 cm of water in the bottom, to allow hydration of the containers' soil. The leaf litter was sprayed from above with deionized water. When both soil and leaf litter were hydrated, the trays with the containers were introduced in two thermostatic chambers (AQUA LYTIC[®]) at constant temperature of 20°C and in dark. Every week a subsample of 20 containers was weighed to assess soil moisture. The amount of deionized water required for attaining field capacity was added to the drainage tray and the leaf litter was sprayed also with deionized water. To avoid heterogeneity of conditions, trays were randomly rotated within and across chambers weekly. The average soil moisture during the experimental period was $81.12\% \pm 1.58 \text{ SE}$ of soil field capacity.

After 62, 111, 161, 253 and 323 days of incubation we collected three randomly-selected containers per treatment (one per drainage tray), with the exception of the last date, when six containers were collected. This decision was made –at the expense of a sixth collection– to compensate for the increased variance of remaining litter mass detected in the later collections. In each container, we gently separated leaf litter from the soil, kept the litter in paper bags and dried it in the oven ($60^\circ\text{C} \geq 48 \text{ h}$). Once dried, the sample was extended on a paper to additionally remove soil particles attached to the litter with a gentle brush before weighing the litter to obtain the proportion of litter mass remaining (LMR). Soils were also kept in paper bags, oven-dried ($60^\circ\text{C} \geq 72 \text{ h}$) and kept in the freezer at -20°C until analysis (Sun et al. 2015). By drying soil samples at a moderate temperature, we minimized the drying period, and then the chances of losing labile organic carbon (Sparling et al. 1985).

Chemical analysis

Leaf litter. From the initial pool of leaf litter we selected 20 g (air-dried weight) per species, keeping the rachis:lamina ratio of full leaves of *Ailanthus* and *Robinia*. From each of the remaining collections, we kept the full amount of remaining litter. Each sample was ground in a Culatti mill to 1 mm- particle size. From the initial pool of leaf litter we collected four pseudo-replicates of 3.5 g for lignin analysis in Ainprot S.A. laboratory (Madrid, Spain), following the method of UNE-EN ISO 13906:2009. N, P and C concentrations of every litter sample were determined with an Automated Wet Chemistry Analyzer (Skalar San++ Breda, The Netherlands) after a digestion with H_2SO_4 and Cu-KSO_4 , in Nutrilab (Rey Juan Carlos I University, Móstoles, Madrid, Spain).

Soils. Soil pH, total N, total C, and N-NO_3^- (which represented 93.4% of the total inorganic N of this soil (Castro-Díez et al. 2012)) were measured at the beginning (day=0, 5 pseudo-replicates), at the middle (day =161, 3 replicates), and at the end of the incubation (day=323, 6 replicates).

Soils were defrosted at ambient temperature, homogenized and ground in a Culatti mill to a 2 mm soil particle. For soil pH assessment, 10 mL of deionized water were added to 5 g of each soil sample, shaken and measured with a pH-meter (Allen et al. 1986). For total N and C, ca. 40 g per sample were digested with H₂SO₄ and Cu-KSO₄, and analyzed as before, with an Automated Wet Chemistry Analyzer (Skalar San++ Breda, The Netherlands). Soil N-NO₃⁻ was extracted by adding 100mL of KCl 2M to 10 g of soil and shaking the mixture for 1 h. After 30 min, 20 mL of clear supernatant were kept in vials and frozen at -20°C before analysis at Nutrilab with an Automated Wet Chemistry Analyzer (Skalar San ++, Breda, The Netherlands).

Statistical analyses

Comparison of litter and soil properties across pure-litter treatments

Initial chemical properties (N, P, C, lignin, C:N and lignin:N), the fraction of initial litter and nutrient (N, P and C) mass that remained in the final collection, and soil properties in the final collection, were compared across the three pure-litter treatments using one-way ANOVA or Kruskal-Wallis when homoscedasticity was not met.

Fitting litter mass loss models

To fit the variation of LMR to time we used two models for each litter treatment: 1) the simple exponential model which assumes that the substrate is completely decomposed at a constant decomposition rate (k).

$$LMR = e^{-kt}, \quad \text{eqn(1)}$$

LMR being the proportion of litter mass remaining, k the decomposition rate and t the time in years (Berg and McClaugherty 2014). Although this is the most widely used model for litter decomposition dynamics, it has been often found to poorly fit observations for late phases of litter decomposition (Berg and McClaugherty 2014). Thus, we fit: 2) the asymptotic model, which assumes that the decomposition proceeds progressively more slowly until approaching to zero, and that a fraction of the litter mass is not decomposed (Berg and McClaugherty 2014).

$$LMR = m + (1-m)e^{-k't/m}, \quad \text{eqn(2)}$$

m being the fraction of litter mass not decomposed at the end of the period (i.e. the model asymptote), k' the initial decomposition rate, and the rest of parameters being the same as before. The best model was considered the one with the lower residual standard error. Model fit was performed with the `nls()` function of the 'stats' package in R.

Expected values of litter mixtures

For each litter mixture and collection day, we calculated the expected litter or nutrient (N, P and C) mass remaining from the values obtained in the pure litter treatments as:

$$MR_t^e(\text{mixture}) = x * MR_t^o(sp_1) + y * MR_t^o(sp_2), \quad \text{eqn(3)}$$

MR_t^e being the expected mass remaining of the litter mixture at time t , MR_t^o the observed mass remaining of the species making up the mixture (sp_1 and sp_2) incubated in isolation, and x and y the proportions of sp_1 and sp_2 in the mixture.

Comparison of observed and expected values

We compared the observed and the expected values of litter (LMR) and nutrient (N, P, C) mass remaining (NMR, PMR and CMR) for every litter mixture treatment and collection date, by subtracting the latter from the former (observed - expected), and assessed whether the value differs from zero using a Student's one-sample t-test.

Effect of incubation time

A two-way ANOVA was applied to assess the effect of incubation time, mixture treatment and their interaction on the deviation of observed from expected values. To increase the statistical power, values of the collections taken at days 62 and 111 (representing the initial phase) were pooled and compared with collections from days 253 and 323 (representing the final phase).

Variation of the effects of non-native litter with its abundance

For all response variables that differ across pure litter treatments in the last collection, we fit the response variable versus the proportion of non-native litter in the mixture using a linear and a quadratic model. These two models were selected to represent different responses after a visual inspection of the data. For each model, we assessed the residual deviance and the Akaike information criterion (AIC). The quadratic model was considered to improve the linear model if its AIC was at least two units lower than that of the linear model (Burnham and Anderson 2002).

Results

Comparison of pure-litter treatments

All litter chemical properties, except for carbon content, differed across species at the beginning of the experiment. The two non-natives, *Ailanthus* and *Robinia*, showed higher N content, lower C:N and lignin:N than the native *Populus*. The P content was the highest in *Ailanthus*, while lignin was the highest in *Robinia*, followed by *Populus* and by *Ailanthus* (Table 1).

Table 1. Initial chemical properties (mean \pm SE) of the three studied species: *Ailanthus altissima*, *Robinia pseudoacacia* and *Populus alba*. The last columns indicate the ANOVA results comparing species. Different letters across columns indicate significant differences ($P < 0.05$).

	<i>Ailanthus</i>	<i>Robinia</i>	<i>Populus</i>	ANOVA F	ANOVA P
Litter N (mg/g)	8.79 \pm 0.77 ^b	9.25 \pm 1.04 ^b	4.66 \pm 0.49 ^a	10.08	0.005
Litter P (mg/g)	0.97 \pm 0.10 ^b	0.38 \pm 0.06 ^a	0.40 \pm 0.05 ^a	22.61	<0.001
Litter C (%)	29.15 \pm 0.72 ^a	27.61 \pm 0.55 ^a	28.25 \pm 0.44 ^a	1.77	0.223
Litter C:N	33.17 \pm 2.81 ^a	29.84 \pm 4.26 ^a	60.67 \pm 5.79 ^b	15.11	0.001
Litter lignin(%)	6.90 \pm 0.26 ^a	11.03 \pm 0.16 ^c	8.78 \pm 0.12 ^b	116.93	<0.001
Lignin:N	8.11 \pm 0.98 ^a	12.45 \pm 1.61 ^a	19.56 \pm 2.26 ^b	11.63	0.003

The decomposition rate obtained with the simple exponential model (k) was the highest for *Ailanthus*, followed by *Populus* and the lowest in *Robinia* (Table 2). Contrastingly, the asymptotic model revealed that the initial decomposition rate (k') followed the opposite rank (*Populus* > *Robinia* > *Ailanthus*, Table 2, Online Resource Fig. S1). However, in contrast to *Ailanthus*, the decomposition rate of *Populus* and *Robinia* litter slowed down quickly, leading to a residue (m) of nearly two thirds of the initial mass (Table 2, Online Resource Fig. S1). According to their lower residual standard error, the asymptotic model gave a better fit of litter decay dynamics for all the treatments (Table 2).

Table 2. Summary of the models applied to fit the litter mass remaining to time (years). For the simple exponential model we show the decomposition rate (k) \pm standard error (SE). For the asymptotic model we show the initial decomposition rate (k') and the proportion of the initial mass that is not decomposed (m). For both models the residual standard error and degrees of freedom (df) are shown.

Treatment*	Simple exponential model			Asymptotic model			
	$k \pm SE$	Residual SE	df	$k' \pm SE$	$m \pm SE$	Residual SE	df
<i>Ailanthus</i>	1.38 \pm 0.11	0.11	35	1.14 \pm 0.44	0.34 \pm 0.05	0.09	34
<i>Robinia</i>	0.40 \pm 0.02	0.05	34	2.49 \pm 0.48	0.73 \pm 0.02	0.03	33
<i>Populus</i>	0.55 \pm 0.05	0.08	34	4.01 \pm 1.10	0.68 \pm 0.02	0.05	33
A25P75	0.66 \pm 0.05	0.09	35	3.73 \pm 0.85	0.63 \pm 0.02	0.05	34
A50P50	0.81 \pm 0.06	0.08	35	1.11 \pm 0.51	0.47 \pm 0.07	0.08	34
A75P25	0.96 \pm 0.06	0.07	35	1.66 \pm 0.35	0.47 \pm 0.03	0.05	34
R25P75	0.49 \pm 0.04	0.07	34	5.16 \pm 1.1	0.72 \pm 0.01	0.03	33
R50P50	0.33 \pm 0.03	0.06	35	2.62 \pm 1.04	0.77 \pm 0.03	0.05	34
R75P25	0.35 \pm 0.03	0.07	35	4.64 \pm 1.38	0.78 \pm 0.01	0.04	34

* Treatments: pure litter of *Ailanthus*, *Robinia* and *Populus*; mixtures between *Ailanthus* (A) or *Robinia* (R) litter and *Populus* litter (P) at 25:75, 50:50 and 75:25%.

Nutrient dynamics during the decomposition of the litter differed across the three species. In the first collection, the fraction of initial N remaining in the litter (NMR) increased above one in the three species, indicating microbial N immobilization. This increase was particularly steep in *Populus*, where the N litter content almost tripled. During the following collections, NMR declined in *Ailanthus*, and remained similar in *Robinia* and *Populus* (Online Resource Fig. S2), leading to a final net N content change of +155% in *Populus*, +54% in *Robinia* and -25% in *Ailanthus* (Table 3). The P content of the litter (PMR) also increased in the first harvest in the three species, again with a steeper increase in *Populus*. Later on, P slightly decreased in *Ailanthus*, kept on increasing in *Robinia* and remained similar in *Populus* (Online Resource Fig. S3), leading to a final net P content change of -4% in *Ailanthus*, +150% in *Robinia* and +187% in *Populus* (Table 3). Finally, the C content of the litter, after an initial slight increase, tended to decline through the remaining collections, leading to a net C loss in the three species, which was the highest in *Ailanthus* (60%), followed by *Populus* (31%) and by *Robinia* (17%) (Online Resource Fig. S4, Table 3).

Table 3. Proportion of litter mass (LMR), N (NMR), P (PMR) and C (CMR) remaining in the litter, and soil properties (total carbon (C), total nitrogen (N), nitrate (N-NO₃⁻) and pH) in the last collection (day=323). Values are means ± SE. The last two columns indicate the ANOVA results comparing species, except for N-NO₃⁻, where the test was a Kruskal-Wallis. Different letters across columns indicate significant differences ($P < 0.05$).

	<i>Ailanthus</i>	<i>Robinia</i>	<i>Populus</i>	ANOVA <i>F</i>	ANOVA <i>P</i>
LMR	0.42±0.05 ^a	0.75±0.01 ^b	0.66±0.02 ^b	27.34	<0.001
NMR	0.75±0.10 ^a	1.54±0.06 ^b	2.55±0.19 ^c	53.19	<0.001
PMR	0.96±0.23 ^a	2.50±0.20 ^b	2.87±0.13 ^b	26.01	<0.001
CMR	0.40±0.05 ^a	0.83±0.03 ^c	0.69±0.02 ^b	35.09	<0.001
Soil C (%)	3.23±0.11 ^a	3.07±0.06 ^a	3.06±0.15 ^a	0.74	0.49
Soil N (mg/g)	2.83±0.11 ^a	2.72±0.08 ^a	2.88±0.10 ^a	0.77	0.48
Soil N-NO ₃ ⁻ (µg/g)	1.12±0.10 ^a	0.78±0.18 ^a	2.03±0.58 ^a	3.62	0.16
Soil pH	7.88±0.02 ^a	7.81±0.03 ^a	0.79±0.05 ^a	1.67	0.22

Soil chemical properties and their dynamics were very similar across treatments (Online Resource Fig. S5-S8). Soil N and soil N-NO₃⁻ were similar at the beginning and at day 161, but in the last collection (day =323) total N slightly increased, while N-NO₃⁻ slightly decreased (Online Resource Fig. S5-S6). Soil C and soil pH showed little variation through the study period (Online Resource Fig. S7-S8). In the last collection, the ANOVA comparing soil properties across pure litter treatments, revealed no significant difference for any of the soil properties (Table 3).

Are there interactive effects?

In all litter mixture treatments, LMR and CMR followed similar patterns, being equal or greater than expected (Figs. 1-2). This indicates additive or antagonistic non-additive effects of mixtures on mass and C decay. For *Ailanthus* mixtures, LMR was larger than expected in the 50:50 mixture for collections taken at days 62, 11 and 253 (Fig. 1), while for *Robinia*, antagonistic effects were only found in the last collection in the 25:75 and the 75:25 mixtures (Fig. 2).

In contrast to LMR and CMR, NMR and PMR in litter mixtures were equal or lower than expected (except for PMR in the last collection of the 50:50 *Robinia:Populus* treatment), indicating additive or synergistic non-additive effects. More synergies were observed in *Ailanthus* mixture treatments – particularly in 25:75 and 50:50 mixtures (Fig. 1) – than in *Robinia* treatments (Fig. 2). Deviations from expected values were greater for PMR than for NMR, indicating stronger interactions among litters for P decay (Figs. 1-2).

Do interactions vary through time?

The two-way ANOVA assessing the effects of collection date (day 62+111 versus day 253+323), mixture treatment and their interaction on the deviation from expected values, revealed a significant effect of collection date only for PMR in the *Ailanthus* mixtures and for LMR and CMR in the *Robinia* mixtures. In the former, all deviations were negative, but larger in the early than in the later phase (Fig. 1, Table 4). In the latter, the deviations from expected tended to turn

from negative or near zero in the early phase to positive values in the later phase (Fig. 2, Table 4). The interaction between collection date and treatment was significant for *Ailanthus* CMR, where the deviation from expected was greater in the early phase for mixtures with low or medium proportion of *Ailanthus*, but the reverse occurred for the 75:25 *Ailanthus:Populus* treatment. In the case of *Robinia*, the interaction was significant for NMR, where the deviation from expected decreased from the early to the late phase in the 25:75 and the 75:25 *Robinia:Populus* treatments, while the reverse occurred in the 50:50 treatment.

394 Table 4. Percentage of deviation of observed - expected values of litter (LMR), N (NMR), P (PMR) and C (CMR) mass remaining in each treatment (T) and
 395 collection date (CD) (mean \pm SE). The 2-way ANOVA columns indicate the significance of the two factors and their interaction for each of the two sets of
 396 litter mixtures (*Ailanthus* + *Populus* on the left, and *Robinia* + *Populus* on the right).

	Harvest	25A-75P	50A-50P	75A-25P	2-way ANOVA	25R-75P	50R-50P	75R-25P	2-way ANOVA
LMR(o-e)	Day 62+111	-1.4 \pm 3.3	10.3 \pm 0.7	2.4 \pm 2.2	T: ns	-2.4 \pm 1.7	5.6 \pm 2.5	-0.5 \pm 2.5	T: *
	Day 253+323	3.2 \pm 1.8	2.8 \pm 3.6	5.0 \pm 2.9	CD: ns	2.2 \pm 1.3	6.4 \pm 2.5	6.2 \pm 1.6	CD: *
					TxCD: ns				TxCD: ns
NMR(o-e)	Day 62+111	-61 \pm 9.6	-31.0 \pm 5.9	-8.9 \pm 9.1	T: ***	-26.9 \pm 3.5	6.5 \pm 8.6	-21.6 \pm 3.3	T: ns
	Day 253+323	-50.2 \pm 7.3	-33.8 \pm 4.8	-6.8 \pm 8.3	CD: ns	-12.3 \pm 9.2	-15 \pm 9.6	2.1 \pm 6.2	CD: ns
					TxCD: ns				TxCD: *
PMR(o-e)	Day 62+111	-104.4 \pm 11.3	-79.7 \pm 9.7	-43.2 \pm 10.8	T: **	-24.9 \pm 12.8	42.1 \pm 24.5	-15.2 \pm 8.3	T: ***
	Day 253+323	-60 \pm 11.3	-49.4 \pm 15.0	-20.7 \pm 9.9	CD: **	2.8 \pm 17.6	53.2 \pm 13.7	14.3 \pm 9.2	CD: ns
					TxCD: ns				TxCD: ns
CMR(o-e)	Day 62+111	4.3 \pm 4.2	18.0 \pm 1.9	0.4 \pm 2.8	T: ns	-0.2 \pm 3.8	8.3 \pm 4.3	-4.6 \pm 5.2	T: ns
	Day 253+323	0.6 \pm 1.7	3.0 \pm 4.0	9.6 \pm 4.4	CD: ns	2.9 \pm 2.1	7.5 \pm 3.2	9.7 \pm 1.7	CD: *
					TxCD: **				TxCD: ns

397 Treatments: mixtures between *Ailanthus* (A) or *Robinia* (R) litter and *Populus* litter (P) at 25:75, 50:50 and 75:25%.

398 Significance of 2-way ANOVA: ns – $P > 0.05$; * – $0.05 \geq P > 0.01$; ** – $0.01 \geq P > 0.001$; *** – $P \leq 0.001$

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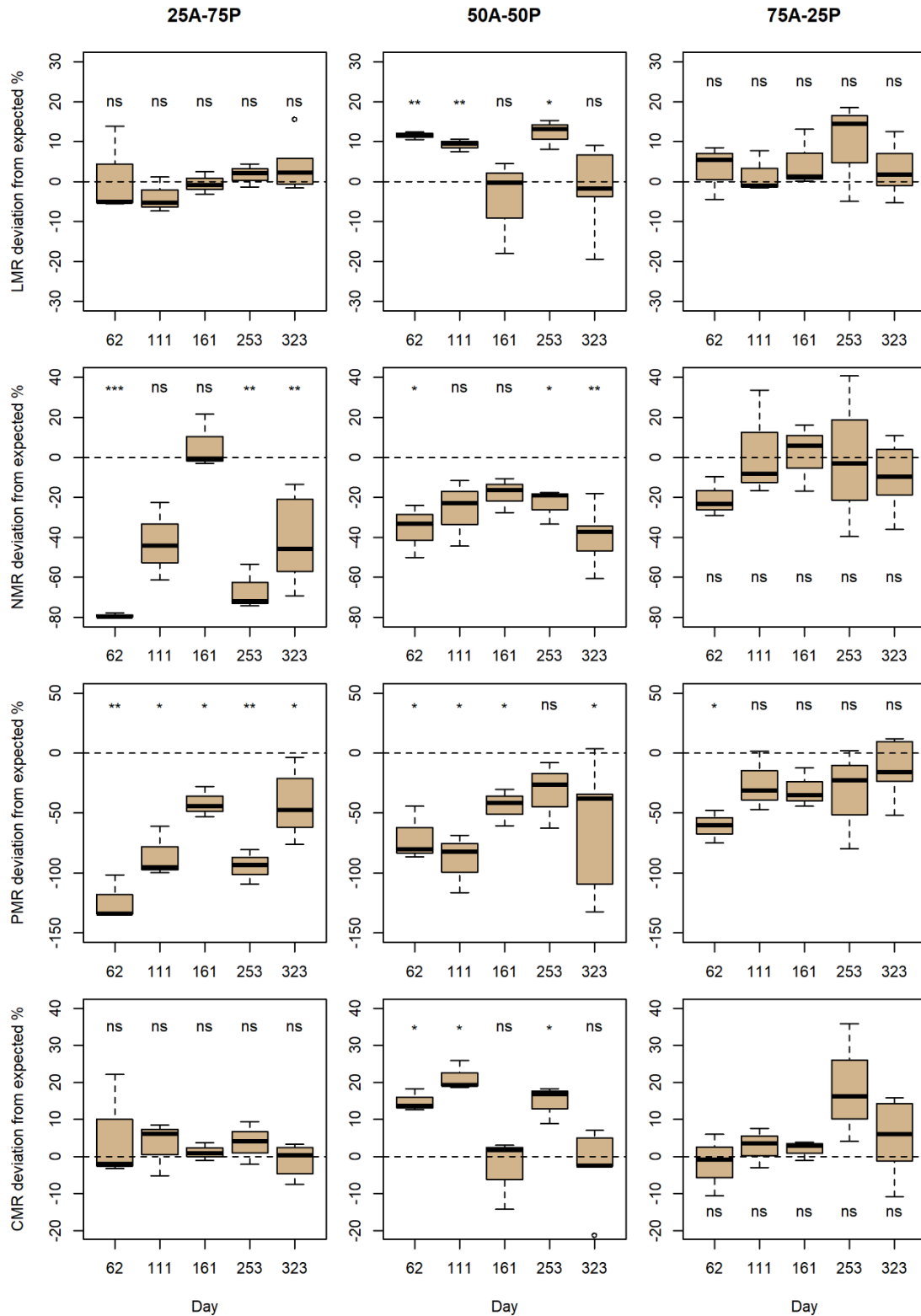
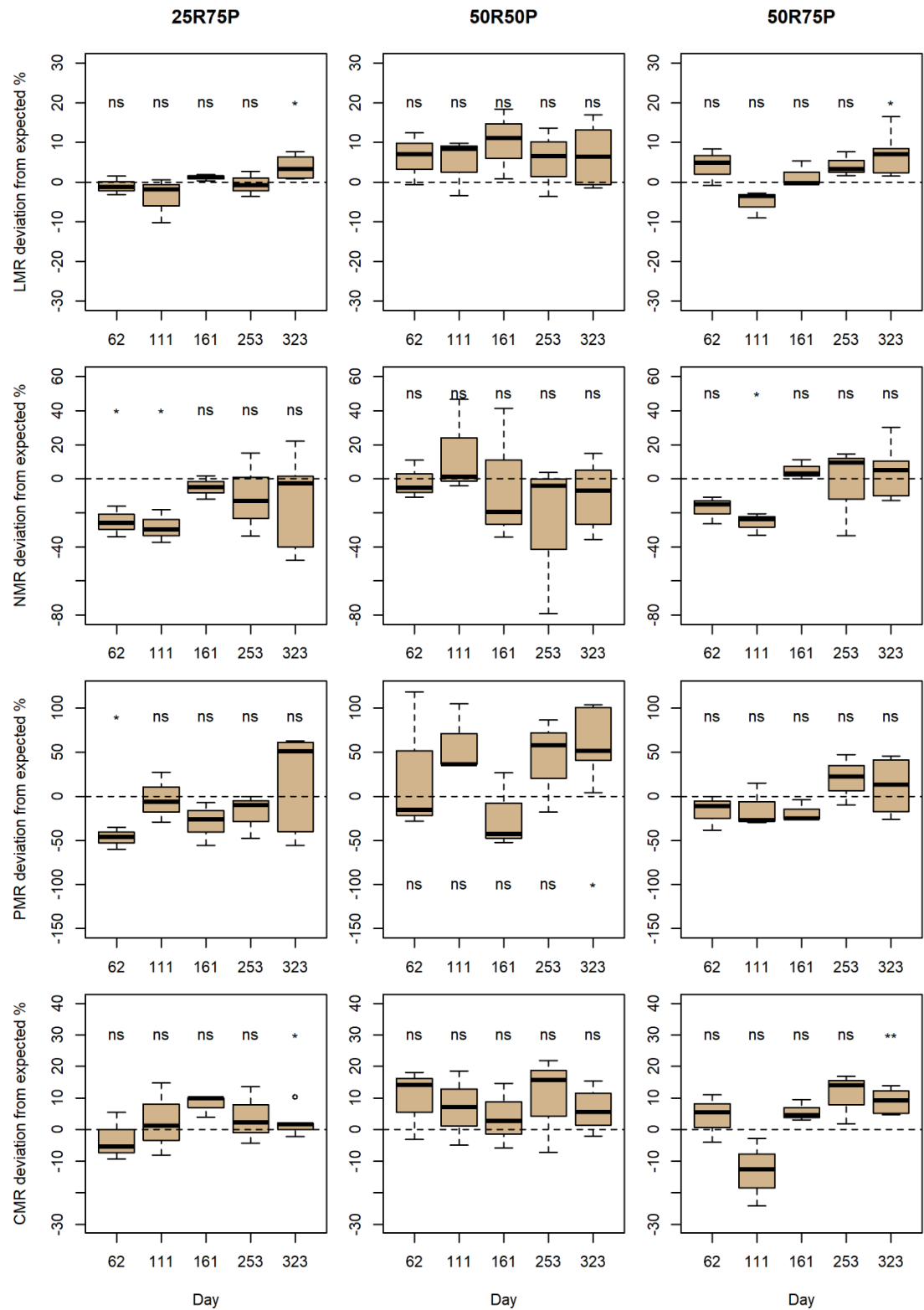


Figure 1. Deviations of litter properties from the values expected under the assumption of additive effects between *Ailanthus* and *Populus* litters in the mixtures. Litter properties were proportions of: initial litter mass remaining (LMR), initial N mass remaining (NMR), initial P mass remaining (PMR) and initial carbon mass remaining in the litter (CMR). Deviations are expressed in percentage. The zero line (where observed an expected values are equal) are represented by a dashed line. Values above zero suggest antagonistic effects, while values below zero suggest

407 synergistic effects. Asterisks indicate significant differences from zero (one-sample *t*-test,
 408 $P < 0.05$)



409 **Figure 2.** Deviations of litter properties from the values expected under the assumption of
 410 additive effects between *Robinia* and *Populus* litters in the mixtures. Litter properties are
 411 abbreviated as in Fig. 1. The zero line (where observed an expected values are equal) are
 412 represented by a dashed line. Values above zero suggest antagonistic effects, while values below

zero suggest synergistic effects. Asterisks indicate significant differences from zero (one-sample *t*-test, $P < 0.05$)

Impact versus abundance of the non-native litter

In the *Ailanthus* mixtures, the two-way ANOVA showed that deviations from expected varied across treatments for NMR and PMR. In both cases, deviations were greater in the mixture where *Ailanthus* was at 25% (Table 4, Fig. 1). In the *Robinia* mixtures, treatment significantly affected the deviations from expected for LMR and PMR. In these cases, deviations were greater in the 50:50 mixtures (Table 4, Fig. 2).

In the *Ailanthus* gradient, the proportion of litter and nutrient mass remaining at the end of the experiment declined at increasing proportion of *Ailanthus* in the mixtures. In the case of LMR and CMR, this decrease was linear to the proportion of *Ailanthus* litter in the mixture (the AIC of the linear model was lower than that of the quadratic model). In the case of NMR and PMR, the quadratic model was the best (AIC at least two units lower than that of the linear model), showing a steeper decrease up to 50% *Ailanthus* litter proportion in the mixture, but a less steep response at higher proportions (Fig. 3).

In the *Robinia* gradient, LMR and CMR in the last collection increased with the proportion of *Robinia* litter in the mixtures, while NMR and PMR decreased (Fig. 3). In this case, only NMR showed a response linear to the proportion of *Robinia* litter. For the remaining variables, the quadratic model showed the best adjustment (AIC at least two units lower than the linear model), with the largest impact (i.e. the largest deviation from the value at 100% native litter) between 50 and 75% of *Robinia* litter in the mixture, and a lower impact with pure *Robinia* litter (Fig. 3).

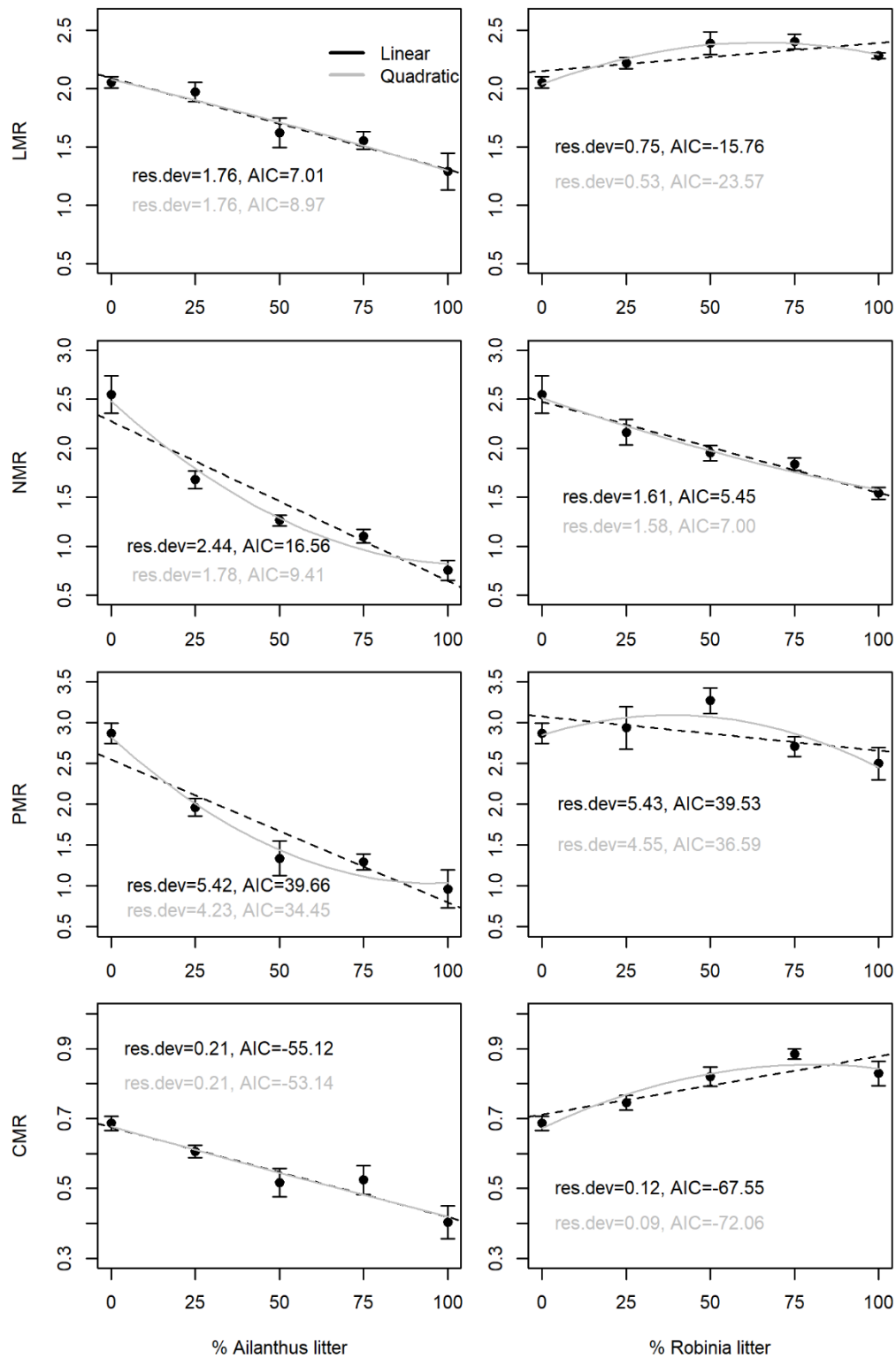


Figure 3. Relation between the response variables (proportion of litter, N, P, and C mass remaining (LMR, NMR, PMR and CMR) in the last collection) and the proportion of the non-native litter in the mixture (*Ailanthus* left and *Robinia* right). Linear (black and dashed line) and quadratic (grey and solid line) model fits, along with the residual deviation (res.dev) and Akaike information criterion (AIC) are shown.

Discussion

Our experiment revealed that the pattern of litter decay varied across species and litter mixture treatments. However, we found little effect of litter types on soil properties at the end of the incubation period. The lack of edaphic macroinvertebrates in our soil sample probably delayed the incorporation of litter-derived material into the soil (Berg and McClaugherty 2014; Hattenschwiler et al. 2005). Also, the presence of high soil organic matter content, derived in this case from native litter, may buffer the effects of the new litter on soil properties in the short term. This result is in line with other studies reporting larger impacts of non-native plants on certain ecosystem processes or community structure than on soil properties (Berendse et al. 1989; Castro-Díez et al. 2009; Castro-Díez et al. 2016; Mack et al. 2001). Thus, the time needed to translate altered litter properties into the soil may be much longer than the time needed for the expression of other impacts (Berendse et al. 1989; Castro-Díez et al. 2009; Marchante et al. 2008; Muñoz Vallés et al. 2011).

How do litter decomposition dynamics differ across the three species?

We found different dynamics of litter and nutrient decay across the three tree species coexisting in riparian forests of central Spain: the native tree *Populus alba* and the two non-native invaders *Ailanthus altissima* and *Robinia pseudoacacia*. The initial litter properties of the invaders (higher N and lower C:N and lignin:N than the native) suggest a faster litter decay, according to previous studies on the litter properties controlling decomposition rates (Aerts 1997; Berg and McClaugherty 2014; Gallardo and Merino 1993). However, this prediction only holds for *Ailanthus*, which showed the smallest values of litter and nutrient mass remaining at the end of the experiment. By contrast, *Robinia* showed litter and nutrient decay patterns similar to those of *Populus*, in spite of its higher initial N content. Previous studies suggested that high initial N content in the litter may accelerate decomposition in the early phase, but retards it in the later phase (Berg 2000; Berg and Ekbohm 1991). This happens because low-molecular N reacts with the increasing fraction of lignin remains, turning them recalcitrant (Berg 2000; Gallardo and Merino 1993; Hattenschwiler and Vitousek 2000). This effect should be stronger in *Robinia* than in *Ailanthus* because of the higher lignin content of the former. In fact, other studies that found a relatively low decomposition rate of *Robinia* litter, attributed it to its high lignin content or lignin:nutrient ratio (Castro-Díez et al. 2009; Medina-Villar et al. 2015a). Our results are in line with recent studies suggesting that invasive species do not necessarily speed up nutrient cycles, as suggested by former reviews (Castro-Díez et al. 2014a; Ehrenfeld 2003; Pyšek et al. 2012), and this general trend may be the result of a publication bias towards the invasive species with the largest impacts (Incerti et al. 2018; Jo et al. 2016).

Are there interactions between non-native and native litter on the decomposition process?

We found different types of effects among the mixed litters across treatments, incubation times, and response variables. Specifically, the sign of the non-additive effects was different depending on the component of the litter considered: LMR and CMR tended to be equal or higher than expected (i.e. additive or antagonistic effects), while the NMR and PMR tended to be equal or lower than expected (i.e. additive or synergistic effects). Most studies assessing changes in N

and C dynamics caused by mixing different litters also report that the responses of both components were not correlated (Hattenschwiler et al. 2005; McTiernan et al. 1997; Quested et al. 2002). In our study, this apparent discrepancy can be explained by the fact that changes in NMR and PMR are due to N and P microbial immobilization, rather than to N and P decay. During the early fast decomposition period (up to day 62), N and P increased in the three litters, but more steeply in the species with faster initial decomposition (i.e. *Populus*, followed by *Robinia*, and *Ailanthus*). This increase suggests that litter mixtures are colonized by the fast-growth opportunistic microorganisms, which retain all N and P from the litter in their biomass, and take up additional N from the surroundings (Berg and McLaugherty 2014; Gallardo and Merino 1992). After 62 days of incubation, N and P leveled off, or even decreased, coinciding with a deceleration of the decomposition rate (Online Resource Fig. S1, S2). Thus, lower NMR and PMR than expected in the mixtures would indicate antagonistic effects on microbial growth, rather than synergistic effects on N and P decay.

Previous reviews found that antagonistic interactions in litter mixtures were less frequent than synergistic interactions (Gartner and Cardon 2004; Lecerf et al. 2011). In our study, the antagonistic effects may be attributed to the relatively high lignin content of the litter of *Robinia* and *Populus*, as compared to *Ailanthus* or other coexisting riparian trees (Alonso et al. 2010; Castro-Díez et al. 2009; Medina-Villar et al. 2015a). As explained above, lignin forms complexes with proteins that are resistant to most decomposing organisms (Berg and McLaugherty 2014; Gallardo and Merino 1993; Hattenschwiler and Vitousek 2000). Thus, the transfer of lignin from *Populus* to *Ailanthus* or the accumulation of lignin from *Robinia* and *Populus* as decomposition proceeds may explain the antagonistic effects detected in some mixtures. The number and strength of the antagonistic effects were higher in *Ailanthus* mixtures, in line with previous suggestions of stronger non-additive effects among the species differing more in their litter properties (i.e. *Ailanthus* and *Populus*) (Quested et al. 2002; Wardle et al. 1997, but see Hoorens et al. 2003).

Do interactions between litters vary throughout incubation time?

Previous studies detected changes in the sign of the interaction among litters throughout the process of litter decomposition and nutrient release (Chen et al. 2013; Gartner and Cardon 2004; McTiernan et al. 1997; Wardle et al. 1997). Litter decomposition has been often suggested to follow two phases (Berg and McLaugherty 2014; Gallardo and Merino 1993). The initial fast-decomposition phase occurs when most soluble and/or labile components are quickly leached or released from the litter, and it is probably driven by early-successional fast-growing bacteria (Berg and McLaugherty 2014; Elgersma and Ehrenfeld 2011). In the later phase, recalcitrant compounds, such as lignin, become dominant, explaining the slowing down of the decomposition (Berg and McLaugherty 2014; Gallardo and Merino 1993). The shift in the ratio of labile versus recalcitrant components in the decomposing substrata is probably accompanied by a shift in the community of decomposers (Berg and McLaugherty 2014; Elgersma and Ehrenfeld 2011; Gallardo and Merino 1993; Lecerf et al. 2011), which may explain changes in the strength or sign of the interactions through time.

In our study, interactive effects between *Robinia* and *Populus* on LMR turned from mostly additive in the early phase (days 62+111) to antagonistic – i.e. larger LMR than expected – in the

later phase (days 253+323). In this case, the shift may be attributed to an extraordinary accumulation of lignin from the two litters through time, as both possessed high initial lignin content. By contrast, the interactive effects between *Ailanthus* and *Populus* litter on PMR (less P immobilization than expected) were stronger in the early than in the later phase. In this case, the growth of early-successional microbes that colonizes *Ailanthus* litter (responsible for P immobilization) may be halted by the formation of recalcitrant bonds between the lignin from the lignin-rich litter of *Populus* and the proteins from the N-rich litter of *Ailanthus* (Berg and McClaugherty 2014; Gallardo and Merino 1993; Hattenschwiler and Vitousek 2000). Thus, shifts in the sign of interactions through time seem to be frequent, the direction of the shift depending on the released component and on the initial composition of the litters in the mixture. These complex interactions make it difficult to derive general conclusions by joining results from experiments performed with different litters and with different time schedules, as highlighted before (Hattenschwiler et al. 2005).

Are the impacts linear to the abundance of the non-native litter?

Most studies assessing the effects of plant invasions compare heavily invaded or monocultures with uninvaded situations (Strayer et al. 2006; Yokomizo et al. 2009). Although this is useful for identifying potential impacts, it does not inform on the impacts of invasions at intermediate abundances. This lack of information often leads researchers or managers to assume a linear relation between the impact and the abundance of the invader (Parker et al. 1999). However, we found in this study that the linear relation was the least frequent, only standing in three out of the eight case studies (two invaders x four response variables). For the remaining cases, the impact (assessed as deviation from the non-invaded situation) increased faster from zero to low or mid abundance, and slower –even leveling off or decreasing– from mid to high abundance. These non-linear responses may be attributed to the fact that the strength of the interactions across litters depended upon the proportion of the invader in the mixtures, being the highest when the invader was 25% (*Ailanthus* impact on NMR and PMR) or 50% (*Robinia* impact on LMR, PMR and CMR). This finding has important implications for the management of these invasive trees in riparian forests of central Spain, and suggests that the most effective reduction of their impacts on litter decomposition would occur at low (*Ailanthus*) or medium (*Robinia*) abundances. By contrast, at high abundance, the benefit of a small reduction of the invasive tree cover will be proportionally smaller (but more costly). Indeed, as previously reported, knowing the shape of the impact-abundance curve is crucial for defining an optimal strategy for the management of invasive plants (Sofaer et al. 2018; Yokomizo et al. 2009).

Limitations of the study and future perspectives

Using microcosm experiments, we have demonstrated that mixing the litter from native and invasive species of central Spain riparian forests may have additive or antagonistic effects on litter decomposition. We have also shown that in most cases the invader's litter impacts are not linear to abundance. Our approach has the advantage of allowing a tight control over environmental conditions, and the use of a standard soil allowed us addressing the potential effect of different litters on soil properties. However, the extent to which these results can be extrapolated to the field remains to be further explored, due to several limitation of our approach. First, our microcosms lack macroinvertebrates. These organisms play a major role on

the litter decomposition, fragmenting the litter and digesting the raw organic matter, facilitating further processing by smaller invertebrates and bacteria (Berg and McClaugherty 2014; Hattenschwiler et al. 2005). The structure and function of the macroinvertebrate community may be altered by the non-native litter (Gutiérrez-López et al. 2014; Medina-Villar et al. 2015a), which in turn may alter the effects that the non-native litter have on the decomposition processes (Hattenschwiler and Gasser 2005). Moreover, some studies revealed that the sign of interactions among litter species may shift depending on the presence or absence of certain macroinvertebrates (Hattenschwiler and Gasser 2005; Hattenschwiler et al. 2005). Second, although here we did not find differential effects of the litter mixtures on soil properties, differences may probably arise in the long term, and such difference might interfere with the litter decomposition process. Third, although our experiment covered an incubation period longer than most decomposition studies, the final proportion of litter mass remaining was still quite high (42-75%), so we cannot provide information about litter interactions in later phases of decomposition. Fourth, we have only observed above-ground effects of the non-native leaf litter, but several studies suggest that the belowground plant inputs (root litter and exudates) have stronger effects on soil processes than above-ground plant inputs (Brant et al. 2006; Keith et al. 2009; Pollierer et al. 2007). Besides, other components of the litter, differing from leaves in physical-chemical properties (inflorescences, fruits, or bark) may represent important inputs of organic matter to the soil in certain periods of the year (Castro-Díez et al. 2014b; Medina-Villar et al. 2015b). Finally, microclimatic conditions in the field vary throughout the seasons, and these variations may interfere with the interactions of the litter mixtures (Aerts 1997; Berg and McClaugherty 2014; Gallardo and Merino 1993). Thus, future research should include macroinvertebrates and other litter components in microcosms and/or combine microcosms with field experiments.

Conclusions

Ailanthus altissima can accelerate the litter decomposition in forests dominated by *Populus alba* due to the production of high-N, low-lignin, fast-decomposing litter. However, the antagonistic effects observed in some litter mixtures might buffer this acceleration. By contrast, *Robinia pseudoacacia* would delay litter decomposition in *Populus* forests, probably due to the high lignin content of its litter. In this case, the delay may be even exacerbated by the antagonistic effects on decomposition observed in litter mixtures, mainly when both litters have similar proportions in the mixture. Effects of non-native litters on soil properties were negligible in the time-scale of this experiment (ca. 1 year). The final effects of the non-native litters on different variables characterizing the decomposition process were non-linear in most cases, with greater changes when the abundance increased from zero to 25 or 50% of the mixture. Impacts leveled off or even decreased at greater abundances. This suggests that control efforts to minimize impacts on litter decay should focus on the earlier or mid stages of the invasions, where the reduction of the non-native tree abundance would have greater benefits. Future studies should include macroinvertebrates in microcosm designs and/or should combine microcosms with field experiments.

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Supplementary Material

This article contains the following Supplementary Material:

Figure S1. Litter mass remaining of pure litter of *Ailanthus altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter mixtures along the incubation

period (*Ailanthus:Populus* litter mixtures at 25:75, 50:50, 75:25 proportions are named 25A-75P, 50A-50P and 75A-25P. *Robinia:Populus* litter mixtures at the same proportions as above are named 25R-75P, 50R-50P, 75R-25P, respectively). The red line represents the exponential model fit, while the blue line represents the asymptotic model.

Figure S2. Proportion of initial nitrogen (N) mass remaining in the pure litter of *Ailanthus altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter mixtures (abbreviations as in Fig. S1) during the incubation period.

Figure S3. Proportion of initial phosphorous (P) mass remaining in the pure litter of *Ailanthus altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter mixtures (abbreviations as in Fig. S1) during the incubation period.

Figure S4. Proportion of initial carbon (C) mass remaining in the pure litter of *Ailanthus altissima* (100A), *Robinia pseudoacacia* (100R), *Populus alba* (100P), and different litter mixtures (abbreviations as in Fig. S1) during the incubation period.

Figure S5. Soil N content in different pure litter and litter mixture treatments (abbreviations as in Fig. S1) during the incubation period.

Figure S6. Soil nitrate content (N-NO₃⁻) in different pure litter and litter mixture treatments (abbreviations as in Fig. S1) during the incubation period.

Figure S7. Soil carbon content (C) in different pure litter and litter mixture treatments (abbreviations as in Fig. S1) during the incubation period.

Figure S8. Soil pH in in different pure litter and litter mixture treatments (abbreviations as in Fig. S1) during the incubation period.